UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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MEMORANDUM

OCT 20 1987

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Dicamba-- One Year Dietary Toxicity Study in Dogs

TO: R. Taylor PM 25
Registration Division (TS-767C)

FROM: K. Clark Swentzel K. Clark Swentzel 9/30 87
Section III
Toxicology Branch (TS-769C)

THRU: Marcia van Gemert, Ph.D. M. van Gemert 10/8/87
Head, Section III
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and

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Chemical: Dicamba (Banvel)

Caswell No.: 295

Registration No.: 876-25

Project No.: 7-0914

EPA ID NO.: 55947-3

An expedited review of the subject study has been requested (Memorandum: Tinsworth, TS-767C, to Barton, TS-769C, August 17, 1987). This study has been evaluated by the Toxicology Branch; the DER is attached.

The ADI committee will determine if the NOEL in this study (2500 ppm/diet; about 52 mg/kg/day) can be used to increase the current ADI (0.0013 mg/kg) which is based on a NOEL (5 ppm/diet; 0.1250 mg/kg/day) from a previous 2-year feeding study in dogs (R. Davis, W. Jolley and K. Stemmer, February 23, 1962. University of Cincinnati, Kettering Laboratory).

Reviewed by: K. Clark Swentzel K. Clark Swentzel, 9/30/87
Section III, Toxicology Branch (TS-769C)
Secondary reviewer: Marcia van Gemert, Ph.D.
Section III, Toxicology Branch (TS-769C) M van Gemert 10/7/87

DATA EVALUATION REPORT

STUDY TYPE: One Year Feeding Study-Dog TOX. CHEM. NO.: 295

ACCESSION NUMBER: 55947-3

TEST MATERIAL: Dicamba (Technical Reference Standard)

SYNONYMS: Banvel

STUDY NUMBER: 163-696

SPONSOR: Sandoz Crop Protection Corp.

TESTING FACILITY: International Research and Development Corp.

TITLE OF REPORT: (Dicamba) One Year Dietary Toxicity Study in Dogs

AUTHOR: M. Blair

REPORT ISSUED: December 19, 1986

CONCLUSIONS:

Dicamba was administered to male and female beagle dogs in the diet for 1 year at nominal levels of 100, 500 and 2500 ppm (mean dosages levels: 2, 11 and 52 mg/kg/day, respectively). The investigated parameters in this study, which included behavior, mortality, bodyweight, food consumption, hematology, serum biochemistry, urinalysis as well as macroscopic and histologic examination of tissues, did not reveal any apparent adverse effect from the test compound. Therefore, the NOEL for dicamba was 2500 ppm in the diet (about 52 mg/kg/day), the highest dosage administered in this test; the absence of any adverse effects among treated animals indicates that the MTD was not attained.

Core-classification: Minimum

Quality assurance: A statement was submitted

Test material

The technical reference standard, dicamba (lot 52625110), which was 86.8% pure, was described as a light tan granular solid. This batch of test material was used throughout the study.

Selection and maintenance of animals

The investigator received 21 purebred beagle dogs/sex from the breeder when the dogs were approximately 4 months old. From these dogs, 16/sex (4/sex/dose level) were subsequently chosen for the test when they were 9 months of age. The weights (apparently at the initiation of the test) were 12.2 to 15.8 kg and 9.5 to 13.8 kg for males and females, respectively. Each dog received appropriate vaccinations and ophthalmologic and complete physical examinations were conducted prior to study initiation.

The dogs were housed individually in metabolism cages and maintained in temperature-(72 ± 1.3°), humidity-(56 ± 8.5%) and light-(12 hour light/ dark cycle) controlled room. Each dog was identified by cage, group and ear tattoo. Water and diet (Certified Canine Diet® #5007, Ralston Purina) were available ad libitum except prior to clinical tests. The basal diet was provided to control animals. Certification analysis of each lot of diet was performed by the supplier and the water supply was analyzed on a quarterly basis for the presence of heavy metals, pesticides and coliform bacteria.

Methods

Diet mixture: preparation, homogeneity and stability

Test material:diet mixtures were prepared at constant concentrations of 0.1, 0.5 and 2.5 g/kg to provide dosage levels of 100, 500 and 2500 ppm, respectively. A premix for each mixture was prepared by grinding the appropriate amount of ground test article with small amounts of ground diet using a mortar and pestle and then mixing in a food mixer for 5 minutes. The resulting premix was then blended with an appropriate additional amount of diet in a twin shell blender for 10 minutes with the intensifier bar operated during the entire blending period. Fresh test article:diet mixtures were prepared each week.

For the determination of test article homogeneity and stability prior to study initiation, 2 batches of test diets were prepared at each required concentration. At the completion of blending, ten stratified 50g samples were collected as the diets were dispensed from the blender. The samples were numbered 1 through 10. Sample number 1 represented the top of the diet and sample number 10 represented the bottom. in addition, a 100g composite sample was collected from each diet, placed in a glass container and stored for 10 days under simulated study conditions.

For the determination of test material concentration, a single 100g composite sample was collected from each treated or control diet each week on the day of preparation and stored frozen. In addition, at

study weeks 1-4 and every fourth week thereafter, duplicate 100g composite samples of the control and treated diets were collected on the day of preparation for analysis of test material concentration.

Observations

Mortality

The dogs were observed for moribundity and mortality at least twice daily throughout the study.

Pharmacotoxic signs

The dogs were observed twice daily for signs of overt toxicity, diarrhea, emesis and inappetence at the time of the moribundity/mortality checks. Detailed observations of appearance and condition, behavior and activity, excretory functions, respiration, orifices, eyes and palpable masses were conducted at least once each week. When red vaginal discharge was noted, that dog was observed daily until the discharge was no longer evident.

Bodyweights

Individual bodyweights were obtained weekly from pretest through 14 weeks of study, once every four weeks thereafter, at week 52 and prior to termination. Bodyweights were inadvertently not obtained at study week 34 as scheduled; therefore, they were obtained at week 35, after which time the original bodyweight was continued.

Food consumption

Same schedule as that for bodyweights without a pretest measurement.

Clinical laboratory parameters

Clinical laboratory studies were conducted on all animals prior to study intiation and at 6 and 12 months of study. Blood samples were obtained from the jugular vein from animals fasted overnight (16-21 hours) with water withheld. Urine was collected during this fasting period.

Hematology

The following hematological measurements were determined on whole blood:

|X| Hematocrit (HCT)
|X| Hemoglobin (HGB)
|X| Leukocyte count (WBC)
|X| Erythrocyte count (RBC)
|X| Platelet count
| | Blood clotting measurements
| | (Thromboplastin time)
| | (Clotting time)
| | (Prothrombin time)

|X| Leukocyte differential count
|X| Mean corpuscular HGB (MCH)
|X| Mean corpuscular HGB conc. (MCHC)
|X| Mean corpuscular volume (MCV)
|X| Reticulocyte count

Blood chemistry

Biochemical measurements, which were determined on serum, included:

Electrolytes:	Other:
|X| Calcium*	|X| Albumin*
|X| Chloride*	|X| Blood creatinine*
| | Magnesium*	|X| Blood urea nitrogen*
|X| Phosphorous*	|X| Cholesterol*
|X| Potassium*	| | Albumin/Globulin ratio
|X| Sodium*	|X| Glucose*
Enzymes	| | Total Bilirubin*
|X| Alkaline phosphatase	|X| Total Protein*
| | Cholinesterase	| | Triglycerides
|X| Creatinine phosphokinase*	| | Serum protein electrophoresis
| | Lactic acid dehydrogenase	
|X| Serum alanine aminotransferase (also SGPT)*	
|X| Serum aspartate aminotransferase (also SGOT)*	
| | gamma glutamyl transferase	
| | glutamate dehydrogenase	

* Required for chronic studies

Urinalysis

Urinalysis determinations included:

|X|Color	|X|Protein
|X|Appearance	|X|Glucose
|X|Microscopic examination	|X|Occult blood
| |of sediment	|X|Nitrite
|X|Specific gravity	|X|Bilirubin
|X|Volume	|X|Ketones
|X|pH	|X|Urobilinogen
|X|Protein	

Pathology

Gross pathology

All animals received a complete postmortem examination under the direct supervision of a pathologist. After an external examination, each animal was opened and the contents of the abdominal, thoracic and cranial cavities were examined both in situ and after removal. Representative samples of designated tissues were collected and placed in phosphate-buffered neutral formalin where appropriate. A full tissue complement was collected from all animals. The following tissues were weighed: adrenal, brain, heart, kidney, liver, ovaries, pituitary, spleen, testes and thyroid/parathyroid complex.

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Histopathology

Representative samples of designated tissues were processed for the preparation and microscopic examination of hematoxylin- and eosin-stained paraffin sections. A full tissue complement was prepared for all animals. The following list constitutes the full complement of tissues:

Digestive system	**Cardiovasc./Hemat.**	**Neurologic**
| | Tongue	|X| Aorta'	|X| Brain (3 levels)
|X| Salivary glands	|X| Heart	|X| Periph. nerve (sciatic)
|X| Esophagus	|X| Bone marrow	|X| Spinal cord (3 levels)
|X| Stomach	|X| Lymph nodes**	|X| Pituitary
|X| Duodenum'	|X| Spleen	|X| Eyes (plus optic nerve)
|X| Jejunum	|X| Thymus	**Glandular**
|X| Ileum	**Urogenital**	|X| Adrenals
|X| Cecum'	|X| Kidneys	| | Lacrimal gland
|X| Colon	|X| Urinary bladder	|X| Mammary gland
|X| Rectum	|X| Testes	|X| Parathyroids
|X| Liver	|X| Epididymides	|X| Thyroids
|X| Gall bladder	|X| Prostate	**Other**
|X|Pancreas	| | Seminal vesicles	|X|Bone (rib)
Respiratory	|X| Ovaries	|X| Skeletal muscle
|X| Trachea	|X| Uterus	|X| Skin
|X| Lung*	| | Cervix	|X| All gross lesions
| | Nose		| | and masses
| | Pharynx		| | Head+
| | Larynx		| | Harderian gland

* with mainstem bronchi
** thoracic (mediastinal), abdominal (mesenteric) and mandibular

Physical examinations

A physical examination was conducted on each dog by a staff veterinarian once during the pretest period and at 3, 6, 9 and 12 months of study. Physical examination of the dog included an inspection for general condition which consisted of examination of the head and neck, thorax, abdomen, external reproductive organs, skin and extremities. Heart and lung sounds were evaluated by percussion and auscultation.

Ophthalmic examinations

An ophthalmoscopic examination was conducted on each dog by a veterinary ophthalmologist once during the pretest period and again prior to study termination. Each eye was dilated with 1-2 drops of a mydriatic (tropicamide, 1.0%). A binocular indirect ophthalmoscope was used to examine each dog. A direct or slit lamp examination was performed when necessary.

Statistical analyses

Body weights, food consumption, clinical laboratory values and organ weights were analyzed using Bartlett's test for homogeneity of variances and analysis of variance (one-way classification). Treatment groups were compared to the control group, by sex, using the appropriate t-statistic (for equal or unequal variances), as described by Steel and Torrie and Ostle. Dunnett's multiple comparison tables were used to judge the significance of differences. Total bilirubin, urine specific gravity and volume were analyzed using a nonparametric approach, by transforming the data to ranks prior to analysis, as described by Conover and Iman. All statistical tests were two-tailed, with $p<0.05$ and $p<0.01$ used as levels of significance.

Results

Mortalities

All dogs survived until the termination of the study.

Pharmacotoxic signs

Inappetence was observed in 2 mid-dose males, 2 high-dose males and 1 high-dose female during the initial weeks of the study. This effect was transient in all dogs except in 1 high-dose male, which is discussed under the food consumption section.

The incidence of emesis was sporadic among treated male groups and all female groups. Diarrhea was observed sporadically among all male and female groups. The frequency of neither emesis nor diarrhea were related to the dosage levels of dicamba. Likewise, the incidence of soft stool was sporadic and apparently unrelated to treatment among females, however, even though soft stool was observed in all male groups, the frequency increased somewhat with the dosage level of dicamba.

Excessive lacrimation was observed in all groups except mid-dose females; there was no apparent treatment-relationship. Likewise, a red vaginal discharge was observed in all females groups and did not appear to be related to treatment.

The investigator indicated that no obvious changes in behavior or appearance were observed that could be attributed to the administration of dicamba, with the exception of inappetence.

Food consumption

As previously indicated, inappetence was observed among treated dogs, especially males, during the initial weeks of the study. One high-dose male consumed a very small amount of the diet mixture during the first 3 weeks of the study (4, 7 and 6 g/day, respectively), therefore, approximately 200 g of the prepared diet was removed from the food hopper and mixed with water to form a slurry. The slurry was offered to this dog for 2 hours, twice each day during weeks 4-6 of the study. Additional test diet mixture was added to the food hopper as required during this interval. The bodyweight of this animal decreased from 14.1 to 10.7 kg during the initial 3 weeks of the study; during week 4 the

bodyweight began to increase and it reached the pre-test level by week 9. This dog was returned to the original test diet mixture at week 7.

There were only sporadic statistically significant ($p<0.05$) differences between test and control food consumption values throughout the study. The investigator provided the average food consumption for the control and each treated group for the entire 52-week test period as follows:

Dosage level (ppm)	Average food consumption, g/dog/day (% difference from control)	
	Male	Female
0	304	278
100	280(-7.9)	267(-4.0)
500	311(+2.3)	279(+0.4)
2500	326(+7.2)	252(-9.4)

Although food consumption during the initial period of the study was somewhat lower than that for controls, apparently because of a palatability problem, the administration of dicamba did not appear to have an overall adverse effect on food consumption. A more definitive evaluation of possible compound-related inhibition of food consumption would have been possible if pre-test consumption values had been determined.

Compound consumption

The investigator determined compound consumption values based on food consumption data and nominal, instead of actual, concentrations of dicamba in the diet. However, since analytical data showed that actual diet-concentrations were frequently above as well as below nominal levels, the values provided by the investigator, although not accurate, are not gross misrepresentations of compound consumption, at any dosage level. The mean proportions of nominal levels (ppm in feed) achieved during the entire study were 98, 96 and 94% for the low, mid and high dosage levels, respectively. Based on these proportions and the food consumption values determined by the investigator, the mean compound consumption levels for the entire study were as follows:

Nominal dietary concentration of dicamba (ppm)	Mean consumption levels of dicamba (mg/kg/day)	
	Males	Females
100	1.99	2.17
500	10.78	11.23
2500	54.99	49.07

Bodyweight

There were no obvious differences in bodyweights between test and control groups during the study with the exception of the mid- and high-dose males in which the mean bodyweights were slightly below the control and pre-test values during the initial weeks of the test, when inappetence was evident. However, none of the observed differences were statistically significant.

It was not possible to determine the potential effects of ingested dicamba on bodyweight gain in this study. Since the dogs were 9 months of age at the initiation of the study, the major portion of the growth phase had been completed before the administration of dicamba.

Clinical studies

Hematology

The RBC parameters (erythrocyte count, hemoglobin level and hematocrit) were lower than control and pretest values in high-dose males at the 6 month interval. Although the difference from control was statistically significant ($p<0.05$), these values were within normal biological variation for this animal and statistically significant differences from control values were not observed for these parameters at the 12-month interval. Therefore, the noted differences in the RBC parameters among high-dose males do not appear to be treatment-related.

All other hematological parameters were comparable between control and treated males and females.

Blood chemistry

Although occasional statistically significant differences were observed between test and control blood chemistry values, there were no obvious trends that would indicate that these differences were related to treatment.

Compared to controls, the only statistically significant different value observed among treated males was the glucose level at 6 months in the mid-dose animals (control-107 and test-97 mg/dl), however, the mean pre-test level for control males was only 79 mg/dl.

Several parameters among treated females were significantly different ($p<0.05$) from the corresponding control values at the 6-month interval only; values for aspartate amino transferase at the high-dose and cholesterol at the mid-dose levels were higher and values for calcium, total protein and globulin in high-dose females were lower than respective control levels.

There were no statistically significant differences between test and control values for biochemical parameters measured at the 12 month interval.

Urinalysis

There were no apparent changes in the measured parameters that were related to the ingestion of dicamba.

Ophthalmic examination

No treatment-related ophthalmic abnormalities were noted.

Physical examination

The investigator indicated that the only change observed in treated animals was the occurrence of occasional small lesions in the roof of the mouth of treated animals at the 100 and 2500 ppm (low and high) dosage levels. The only reference to these lesions at termination was a description of a soft palate ulcer in 1 high-dose female during the macroscopic examination.

Pathology

Gross pathology

Splenic congestion was observed in 2 mid-dose females and 4 high-dose males. The investigator implied that this was due to the procedure used for euthanasia in which the dogs were administered sodium pentobarbital intravenously and then exsanguinated. Congestion would occur if the dog died before blood had drained completely from the spleen. The investigator's explanation is plausible.

Organ weights

None of the observed differences in absolute and relative organ weights for control and treated dogs were statistically significant, nor did they appear to be related to the administration of dicamba. Increased spleen weights among mid-dose females and high-dose males were related to the congestion described above as follows:

Relationship between spleen weights and splenic congestion (NC,F,M)*

Control (NC)		Test (dicamba)	
Male (g)	Female	Male (2500 ppm) (g)	Female (500 ppm)
67.84	43.55	41.98 (F)	30.18 (NC)
42.20	21.23	144.72 (M)	29.45 (NC)
37.46	55.69	157.11 (M)	119.05 (M)
34.30	35.67	115.73 (M)	33.04 (F)
Mean (S.D.)			
45.45 (15.28)	39.04 (14.45)	114.89 (51.60)	52.73 (44.11)

* NC: no congestion; F: focal; M: multifocal.

These data indicate that the noted increases in spleen weights could be attributed to multifocal congestion.

Histopathology

Other than splenic congestion, the only noteworthy difference observed between control and treated animals was the occurence of interstitial pneumonia in treated females (3 in the 500 and 1 in the 2500 ppm groups, respectively).

The observed histological differences between tissues from control and test animals did not reveal any trend or dose-response relationship indicative of a treatment-related effect.

Discussion

Adverse effects from the ingestion of dicamba in the diet for 1 year were not apparent in the investigated parameters at any dosage level in this study, therefore, the MTD was not attained. The administration of higher dietary levels would probably not have been practical since inappetence was induced at the 500 and 2500 ppm levels, especially in 1 male receiving 2500 ppm, during the initial phase of the study. However, higher dosages could have been achieved by administering dicamba in gelatin capsules. No other behavioral changes were noted among treated dogs.

Emesis, soft stool or diarrhea were observed sporadically in all groups and both sexes, however, the incidence of soft stool increased slightly with increased dietary levels of dicamba in males.

Differences in food consumption and bodyweight values were not remarkable following the initial period of inappetence.

The investigator provided compound consumption data based on mean food consumption values and nominal dietary concentrations of dicamba. Compound consumption based on mean food consumption values and data from analyses of the compound-diet mixtures showed that males and females in the 2500 ppm groups consumed mean dosages of approximately 55 and 49 mg dicamba/kg/day, respectively, during the 52-week test period.

The mean values for erythrocyte count, hemoglobin level and hematocrit, among males in the 2500 ppm group, were depressed in comparison to corresponding control values at the 6 month interval. These changes do not appear to be treatment-related since the depressed values were within normal biological variation for dog and differences from control values were not observed at the 12-month interval.

Periodic differences between test and control blood biochemistry or urinalysis values had no apparent relationship with treatment. Elevated mean spleen weights among 500 ppm females and 2500 ppm males, in comparison to respective controls, were associated with multifocal splenic congestion. This difference can probably be attributed, as postulated by the investigator, to incomplete drainage of blood from the affected spleens during the euthanasia procedure.

The histologic examination revealed interstitial pneumonia in 3 females in the mid-dose and 1 female in the high-dose groups. The only other noteworthy difference observed between test and control animals was splenic congestion. There was no conclusive evidence that any of the noted histologic differences between test and control dogs were treatment-related.

Conclusions

Dicamba was administered to male and female beagle dogs in the diet for 1 year at nominal levels of 100, 500 and 2500 ppm. The investigated parameters in this study, which included behavior, mortality, bodyweight, food consumption, hematology, serum biochemistry, urinalysis as well as macroscopic and histologic examination of tissues, did not reveal any apparent adverse effect from the test compound. Therefore, the NOEL for dicamba was 2500 ppm in the diet (approximately 52 mg/kg/day), the highest dosage administered in this test. The MTD was not reached in this study.

It was not possible to detect potential inhibition of bodyweight gain from ingested dicamba in the present study since the dogs were 9 months of age at the intiation of the study. The use of dogs 4-6 months old would have exposed the animals to test compound during the rapid growth phase of maturation.